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SIMPLE AND EXTRACTION-FREE SPECTROPHOTOMETRIC METHODS FOR RISPERIDONE IN PURE FORM AND IN DOSAGE FORMS

Two simple, sensitive and extraction-free spectrophotometric methods are described for the estimation of risperidone (RSP) in both pure and in pharmaceutical preparations. The proposed methods are based on the formation of ion-pair complex between RSP and the dyes bromophenol blue (BPB) in method A and phenol red (PR) in method B, at room temperature, to form yellow colored products which show maximum absorbance at 410 and at 400 nm in methods A and B, respectively. Beer's law was obeyed in the concentration range of 0.5-10 and 0.5-25 μg mL^{-1} in methods A and B with apparent molar absorptivities of 3.44×10^4 and 0.85×10^4 L moL^{-1} cm^{-1}, respectively. The limit of detection for method A is found to be 0.0056 and for method B is 0.132 μg mL^{-1}. The composition of the ion-pairs was established by Job's method and it was found to be 1:1 for both the methods A and B. The proposed methods have been applied successfully to the determination of RSP in pharmaceutical preparations. The results were statistically compared with those of a reference method by applying the Student's t-test and F-test. The methods developed were validated for accuracy and precision by performing recovery experiments via standard addition technique.

Keywords: ion-pair; spectrophotometry; risperidone; pharmaceutical preparations.

Risperidone (RSP) is an atypical antipsychotic drug with a relatively low incidence of extrapyramidal side effects. It is used for the treatment of schizophrenia, bipolar disorder and behavior problems in people with autism. Chemically, it is 4-[2-[4-(6-fluorobenzo[*d*]isoxazol-3-yl)-1-piperidyl]ethyl]-3-methyl-2,6-diazabicyclo[4.4.0]deca-1,3-dien-5-one (Figure 1). In 2003, the FDA approved RSP for the short-term treatment of the mixed and manic states associated with bipolar disorder. It is also approved in 2006 European Pharmacopoeia for the treatment of irritability in children and adolescents with autism. The drug is official in 2005 European Pharmacopoeia and the official method of its determination is high-performance liquid chromatography [1].

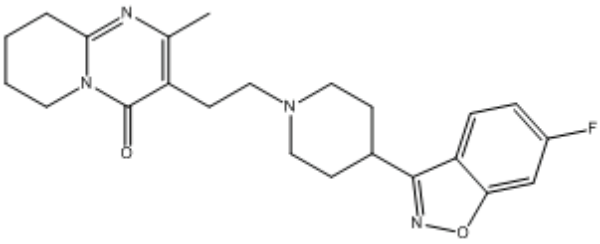

Figure 1. Structure of Risperidone.

Several methods have been used to determine RSP in biological samples including HPLC with mass spectrophotometric detection [2], HPLC with electrochemical detection [3-4], RPHPLC with UV detection [5], electrophoresis [6] and MEPS-LC-UV [7]. The most extensively used technique for its determination is LC-MS/MS but, several procedures using this technique are confined to biological fluids like human

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plasma [8-11] and urine [12] and serum [13]. A limited number of analytical methods for the quantitative estimation of RSP in pharmaceutical samples are known. Procedures based on high performance liquid chromatography and thin layer densitometric methods [14], RPHPLC [15-16], chemiluminescence assay [17], spectrophotometry [18-21] and gas chromategraphic [22] methods are available in the literature. The reported chromatographic techniques [14-16], require expensive experimental set-up. UV-spectrophotometric [18,19], extractive colorimetric [20], and indirect spectrophotometric [21] methods were utilized for its determination. In UV-spectrophotometric methods [18,19], 0.1 N HCl was used as a solvent. In extractive colorimetric methods [20] methyl orange, orange G and cobalt thiocyanate were used as ion-associating agents with the drug and the formed ion-associated complexes were extracted into an organic solvent. In an indirect method [21], the unreacted oxidant chloramine-T decolorizes the dyes, Xylene cyanol FF and Malachite green. Thus, there is a need to develop sensitive, accurate and cost-effective methods for its determination.

The aim of the present investigation is to develop simple, sensitive and economically viable methods that could be used to determine RSP in bulk drug and in pharmaceutical dosage forms. The proposed methods are based on the formation of ion-pair complexes between RSP and sulphonphthalein dyes, namely, bromophenol blue (BPB) and phenol red (PR).

EXPERIMENTAL

Apparatus. All absorbance measurements were performed using a Systronics Model 166 digital spectrophotometer provided with 1-cm matched quartz cells.

Reagents and materials. All chemicals and reagents used were of analytical reagent grade and HPLC grade organic solvents were used throughout the investigation.

1. *Bromophenol blue (BPB, 0.1%).* 0.1 g of BPB (B. D. H. Ltd., Poole, England) was dissolved in 5 mL of acetone (S. D. Fine Chem. Ltd., Mumbai, India, dye content 95%) and made up to 100 mL with acetone.
2. *Phenol red (PR, 0.1%).* 0.1 g of PR (B. D. H. Ltd., Poole, England) was dissolved in 5 mL of acetone (S.D. Fine Chem. Ltd., Mumbai, India, dye content 95%) and made up to 100 mL with acetone.
3. *Standard RSP solution.* Pharmaceutical grade RSP certified to be 99.99% pure and it was received from Cipla India Ltd., Mumbai, India, as a gift and was used as received. A stock standard solution equivalent to 100 μg mL^{-1} of RSP was prepared separately by dissolving 10 mg of the pure drug in 100 mL dichloromethane (Qualigens Fine Chem, Mumbai, India) in method A and in 100 mL 1,4-dioxane (Merck, Mumbai, India) in method B. Working solutions were prepared as required by dilution.

A pharmaceutical formulation of RSP such as respidon (Torrent (Mind)) was purchased from local markets.

General procedures

Calibration curve - method A

Aliquots of standard solution containing 0.25--5.0 mL (20 μg mL^{-1}) of RSP were transferred into a series of 10 mL calibrated flasks. To this solution, 1 mL 0.1% BPB was added, and the contents were diluted to the mark with dichloromethane and mixed well. The absorbance of the yellow colored ion-pair complex was measured after 10 min at 410 nm against the reagent blank prepared similarly, but without drug content at room temperature. The amount of RSP in the pure sample and also in the drug formulations was computed from the concurrent calibration curve or the regression equation.

Calibration curve - method B

Aliquots of a standard drug solution ranging from 0.5-25 μg mL^{-1} were taken in a series of 10 mL calibrated flasks. Then, to each calibrated flask 2 mL of 0.1% phenol red was added. The contents were diluted to the mark with 1,4-dioxane, mixed well and the absorbance of the yellow colored product was measured after 10 min at 400 nm against the reagent blank at room temperature. The amount of RSP present in the sample was computed from calibration curve or the regression equation.

Procedure for tablets

Twenty tablets were ground into fine powder and quantity of the powder equivalent to 10 mg of RSP was weighed accurately into a separate 100 mL calibrated flasks and 10 mL each of the dichloromethane for method A and 1,4-dioxane for method B were added. The content was shaken for about 30 min; the volume was diluted to the mark with suitable solvents and mixed well and filtered using a Whatman No. 41 filter paper. The filtrate containing RSP was at a concentration 100 μg mL^{-1} was subjected to analysis by the procedure described above after suitable dilution step.

RESULTS AND DISCUSSION

Both the methods involve the formation of ion-pair complexes between risperidone (RSP) and acid dyes, bromophenol blue (BPB) and phenol red (PR)

with absorption maxima at 410 and 400 nm for BPB and PR, respectively. The absorption spectra of these complexes such as RSP-BPB and RSP-PR are presented in Figures 2a and 2b, respectively.

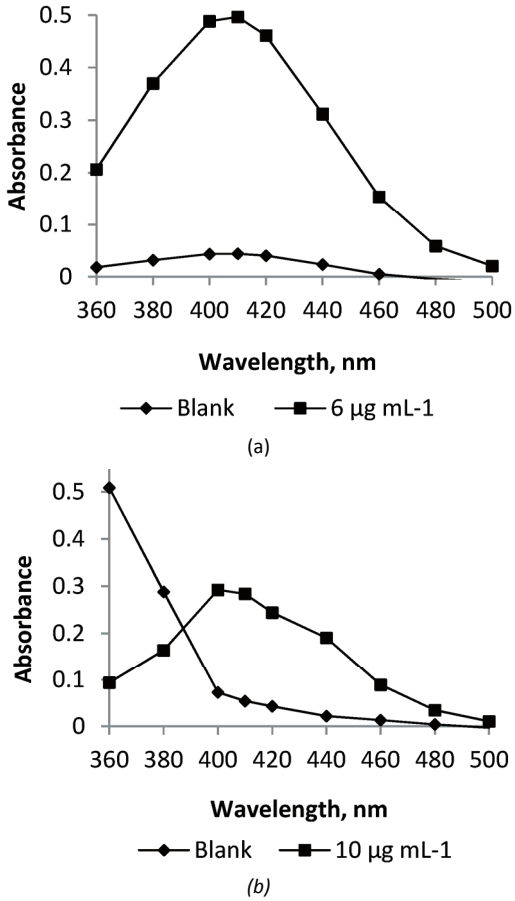


Figure 2. Absorption spectra for a) RSP-BPB and b) RSP-PR.

Chemistry of the method

Basic nature of the drug RSP utilizes the anionic dye to form an ion-pair complex. Due to resonance effect, the protonation of benzisoxazole ring and in pyrimidin-4-one is very difficult. Thus, there is only one site which is susceptible to protonation and that is the nitrogen in the piperidine ring [23]. An intense yellow colored ion-pair formed is due to the opening of lactoid ring and subsequent formation of quinoid group. Among the two tauatomers present in equilibrium, a quinoid group must predominate since the strong acidic nature of the sulfonic group. Finally, protonated RSP forms ion-pairs with the dyes BPB and PR in 1:1 ratio. The possible reaction pathway is depicted in Schemes 1 and 2.

Optimization of variables

Several experimental parameters were studied by measuring the absorbance of the ion-pair complex between RSP and BPB at 410 nm, and RSP-PR at 400 nm to establish rapid, sensitive and stable methods.

Effect of solvent

The choice of solvents for the formation of ion-pair complex was studied. Acetonitrile, dichloromethane, chloroform, 1,4-dioxane, carbon tetrachloride, methanol, ethanol and 2-propanol were tested. Better results were obtained when RSP was dissolved in dichloromethane and 1,4-dioxane in methods A and B, respectively. The other solvents yielded low sample absorbance values or higher blank absorbance values. Among the solvents studied, dichloromethane and 1,4-dioxane were found to be the best and thus selected for the experimental studies.

Effect of dye concentration

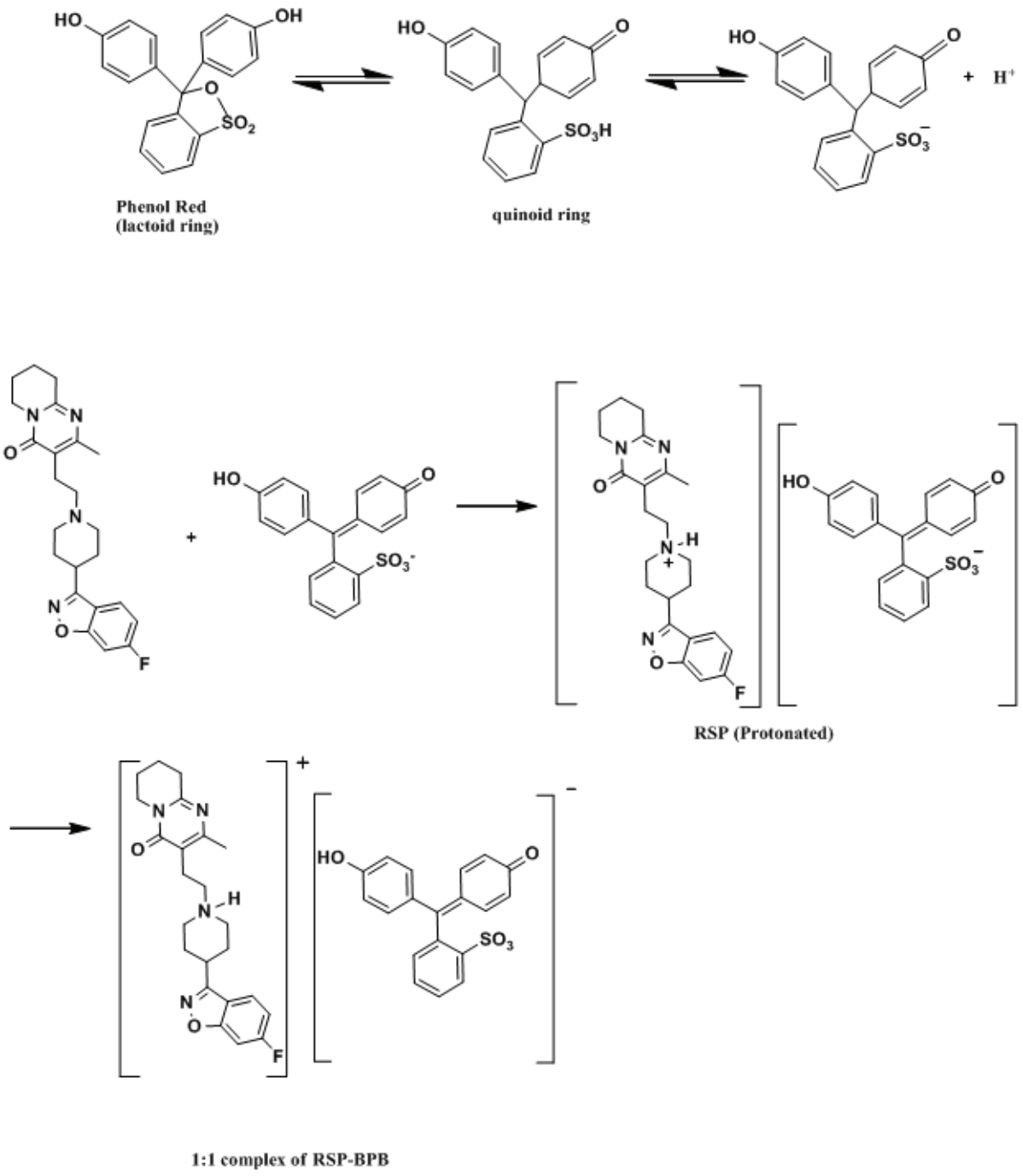
To study the effect of concentration of different reagents such as BPB or PR for the formation of the ion-pair complex varying amounts of cited reagents (0.5-3.0 mL of 0.1% BPB or 1.0-3.0 mL of 0.1% PR) was mixed with RSP (10 µg mL^{-1}). A volume of 1 mL of 0.1% BPB and 2 mL of 0.1% PR reagent solutions in a total volume of 10 mL were found to be sufficient in methods A and B, respectively.

Effect of reaction time and stability of the ion-pair complex

After the addition of dye (0.1% BPB or PR), the effect of standing time was also studied. It was found that 10 min standing time was sufficient for the complete formation of the ion-pair complex in both the methods. At room temperature, the absorbance of the formed ion-pair complex was stable for a period of 2.5 and 2 h in methods A and B, respectively.

Stoichiometry

The Job's method of continuous variation [24] of equimolar solutions was used to establish the stoichiometry of the resulting ion-pair products. The solutions equivalent to 6.09×10^{-5} and 1.22×10^{-4} M RSP was prepared in dichloromethane in (method A) and in 1,4-dioxane (method B), respectively. Further, 6.09×10^{-5} M BPB and 1.22×10^{-4} PR, solutions were prepared in acetone. A series of solutions were mixed in complimentary proportions. In method A, the volume was completed up to the mark using dichloromethane and with 1,4-dioxane in method B. The absorbance of the resulting ion-association complex was measured at their respective wavelengths (λ_{max}) against the reagent blank prepared under similar conditions. The Job's method of continuous variations graph for the reaction between RSP and BPB or PR (Figure 3) shows that the interaction occurs on an equimolar basis *via* the formation of a ion-pair complexes in the ratio 1:1 (RSP:BPB or PR).



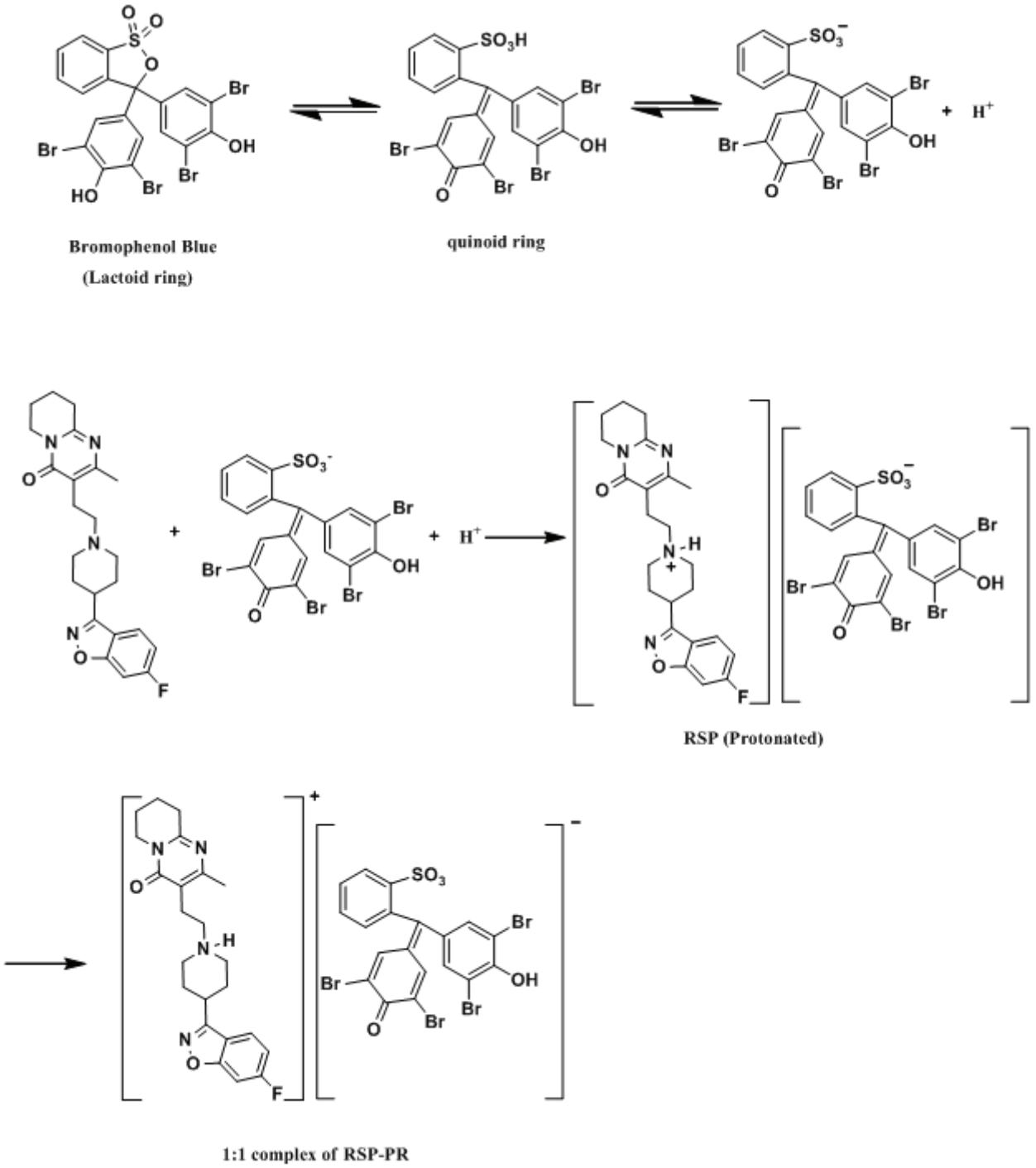
Scheme 1. Possible reaction pathway for the formation of RSP-PR ion-pair complex.

Method validation

According to the ICH guidelines [25], all the methods were validated for linearity and sensitivity, limit of detection (LOD) and limit of quantitation (LOQ), precision, accuracy, selectivity and recovery.

Linearity, sensitivity, limits of detection and quantitation

Under established experimental conditions for both the methods A and B, a linear correlation was found between the absorbance at respective wavelengths and concentration of RSP in the ranges are given in Table 1. Regression analysis of the calibration curve using the method of least-squares was made to calculate the slope (*b*), intercept (*a*) and correlation co-efficient (*r*) for each method (methods A and B) and the values are presented in Table 1. The optical characteristics such as absorption maxima, Beer's law limit, molar absorptivity and Sandell's sensitivity values of both the methods are also



Scheme 2. Possible reaction pathway for the formation of RSP-BPB ion-pair complex.

given in Table 1. The calibration curves of the methods A and B are given in Figures 4 and 5, respectively.

The limit of detection (LOD) and limit of quantification (LOQ) evaluated as per ICH guidelines using the formulas:

$LOD = 3.3\sigma/s$

$LOQ = 10\sigma/s$

where σ is the standard deviation (n = 5) of reagent blank determination and s is the slope of the calibration curve.

Precision and accuracy

The intra-day and inter-day precision and accuracy of the methods developed were evaluated by replicate analysis of drug samples at three different concentrations (low, medium and high) (Table 2)

within the working limits, each being repeated five times. The relative error, *RE* (%) and relative standard deviation, *RSD* (%) values of both intra and inter-day studies were satisfactory and showed that the best appraisal of the procedures in daily use. The analytical results obtained from this investigation are summarized in Table 2. The values percentage relative error between the concentrations of RSP for taken and found showed the high accuracy of the methods. The results obtained are presented in Table 2 and show that the accuracy is good.

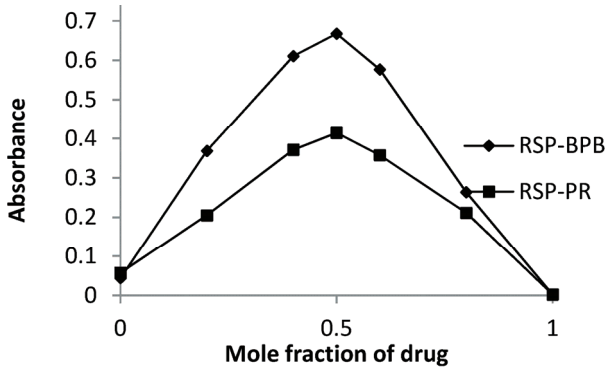


Figure 3. Job's plot for RSP-BPB and RSP-PR ion-pair complex.

Application to analysis of pharmaceutical samples

To check the validity of the proposed methods, RSP determined in respidon tablets and the results are presented in Table 3. The results of an assay of respidon were statistically compared with the reference method [18] by applying the Student's-*t* test for accuracy and *F*-test for precision. The results in the Table 3 showed that there is no significant difference between the proposed and reference method [18] at the 95% confidence level with respect to accuracy and precision. The calculated *t*- and *F*-values (Table 3) did not exceed the tabulated values (t = 2.77 and F = = 6.39).

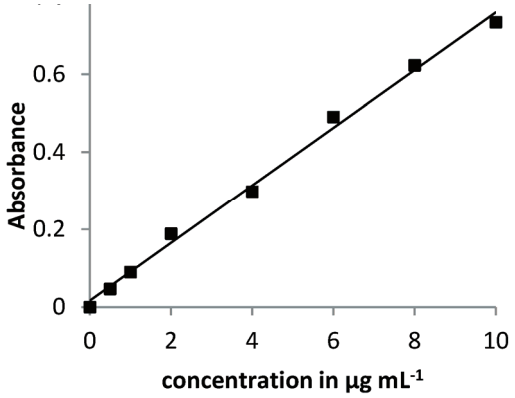


Figure 4. Calibration curve for RSP using BPB.

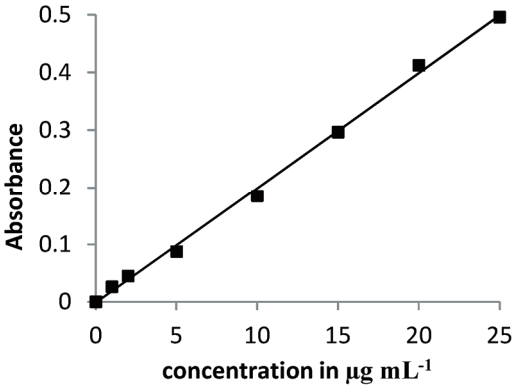


Figure 5. Calibration curve for RSP using PR.

Recovery study

The accuracy and precision of the proposed methods were further ascertained by performing recovery studies. Pre-analyzed tablet powder was spiked with pure drug at three different concentrations and the total was found by the proposed methods. Each

Table 1. Analytical and regression parameters of the proposed methods; y = a + bx, where x is the concentration of RSP in µg mL^{-1} and y is the absorbance at the respective λ_{max}, S_a is the standard deviation of the intercept, S_b is the standard deviation of the slope

Parameter	Method A	Method B
λ_{max} / nm	410	400
Linear range, µg mL^{-1}	0.5-10	0.5-25
Molar absorptivity (ε), L mol^{-1}cm^{-1}	3.44×10^4	0.85×10^4
Sandell sensitivity, µg cm^{-2}	0.0119	0.0482
Intercept (a)	0.0151	-0.0015
Slope (b)	0.0750	0.0200
Correlation coefficient (r)	0.998	0.9988
S_a	0.0274	0.0143
S_b	0.0028	0.0006
LOQ / µg mL^{-1}	0.0169	0.4000
LOD / µg mL^{-1}	0.0056	0.1320

Table 2. Evaluation of accuracy and precision; RE: relative error; RSD: relative standard deviation

Method	RSP taken, μg mL^{-1}	Intra-day accuracy and precision			Inter-day accuracy and precision		
		RSP found[a],μg mL^{-1}	*RE* / %	*RSD* / %	RSP found[b], μg mL^{-1}	*RE* / %	*RSD* / %
A	2	2.02	-0.74	0.34	2.04	-1.94	0.55
	4	3.97	0.73	0.71	4.03	-0.74	0.75
	8	7.98	0.27	0.38	8.10	-1.27	0.46
B	5	4.98	0.37	0.52	5.02	-0.48	0.45
	10	9.96	0.38	0.59	10.04	-0.39	0.54
	20	19.88	0.61	0.46	20.08	-0.40	0.43

[a]Mean value of 5 determinations; [b]mean value of 3 determinations

Table 3. Results of determination of RSP in tablets and statistical comparison with the reference method; tabulated t - and F-values at 95 % confidence level are 2.77 and 6.39, respectively

Tablet brand name	Nominal amount, mg per tablet	Found[a], % of nominal amount±*SD*		
		Reference method [18]	Method A	Method B
Respidon[b]	1 mg	102±0.188	101.06±0.0.40	101.54±0.43
			t = 2.53	*t* = 1.19
			F = 4.60	*F* = 5.18

[a]Mean value of five determinations; [b]Torrent (Mind)

determination was repeated three times. The recovery of the pure drug added was quantitative and revealed that frequently encountered common ingredients of formulations were found not to interfere. The results of recovery study are compiled in Table 4.

trol and routine estimation of drug in pharmaceutical tablets where precision, time and cost effectiveness of analytical methods are important.

Table 4. Results of recovery experiments via the standard addition technique

Tablet brand name	Method A				Method B			
	RSP tablet μg mL^{-1}	Pure RSP added μg mL^{-1}	Total found μg mL^{-1}	Pure RSP recovered±SD[a] %	RSP tablet μg mL^{-1}	Pure RSP added μg mL^{-1}	Total found μg mL^{-1}	Pure RSP recovered ± *SD*[a] %
Respidon (Torrent (Mind))	2	2	4.023	101.17±0.52	5	5	10.06	101.20±0.85
	2	4	6.061	101.52±0.64	5	10	14.99	99.93±0.32
	2	6	8.029	100.49±0.22	5	15	20.24	101.62±0.21

CONCLUSION

In the present study, RSP forms ion-pair complex with BPB or PR at room temperature. These methods do not involve multi steps and do not take more operator time (not more than 10 min). These methods are simple and not required expensive experimental set up like HPLC and other chromatographic methods. In terms of simplicity, rapidity, sensitivity and free from interference by common additives and excipients, the reagents utilized in the proposed methods are cheaper, readily available and the procedures do not involve any critical conditions or tedious sample preparation. The proposed methods are sensitive enough to determine small amounts of the drug; therefore, both methods can be used for quality con-

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NAUČNI RAD

JEDNOSTAVNE DIREKTNE SPEKTROFOTOMETRIJSKE METODE ZA ODREĐIVANJE RISPERIDONA U ČISTOJ FORMI I U FARMACEUTSKIM OBLICIMA

U radu su razvijene dve jednostavne, osetljive, direktne spektrofotometrijske metode za koje nije potrebno vršiti ekstrakciju aktivne komponente za određivanje risperidona (RSP) u čistoj formi i farmaceutskim preparatima. Metode su zasnovane na reakciji formiranja kompleksa uz izmenu naelektrisanja (jonski par) između RSP i boja bromfenolplavo (BPB) u metodi A i fenol crveno (PR) u metodi B na sobnoj temperaturi, pri čemu se grade žuto obojeni kompleksi, čiji se intenziteti mere na 410 nm u metodi A i na 400 nm u metodi B. Nađeno je da važi Berov zakon u opsegu koncentracija od 0,5 do 10 μg ml^{1} u metodi A, i u opsegu koncentracija od 0,5 do 25 μg ml^{1} u metodi B. Molarne apsortivnosti kompleksa su 3,44×10^{4} L moL^{-1} cm^{-1}u metodi A i 0,85×10^{4} L moL^{-1} cm^{-1} u metodi B. Nađeno je da je limit detekcije za metodu A 0,0056, a 0.132 μg mL^{-1}za metodu B. Jobovom metodom je određeno da je sastav formiranih kompleksa 1:1. Predložene metode su uspešno primenjene za određivanje RSP u faramceutskim preparatima. Rezultati su statistički upoređeni sa referentnom metodom primenom Studentovog t testa i F testa. Razvijene metode su validirane. Tačnost i preciznost metode je potvrđena metodom standardnog dodatka sa odličnim „recovery" vrednostima.

Ključne reči: jonski par, spektrofotometrija, risperidon, farmaceutski preparati.